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Anti-malarial activity of N^6-modified purine analogues

Kathleen Too,[a] Daniel M. Brown,[a] Emily Bongard,[b] Vanessa Yardley,[b] Livia Vivas[b] and David Loakes[a,*]

[a]*Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK*
[b]*London School of Hygiene and Tropical Medicine, Department of Infectious and Tropical Diseases, Keppel street, London WC1E 7HT, UK*

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Abstract—*Plasmodium falciparum* causes one of the deadliest forms of malaria and resistance to the currently available drugs makes it imperative to develop new, safe and potent drugs. Parasites such as *P. falciparum* are unable to synthesise purines de novo and to this end often have multiple purine uptake and salvage systems. With this in mind, we have designed and synthesised libraries of purine analogues as potential anti-malarial agents. Herein, we report three compounds with promising activity against the highly chloroquine-resistant VS1 *P. falciparum* namely: N^6-hydroxyadenine (**1c**), 2-amino-N^6-aminoadenosine (**2b**) and 2-amino-N^6-amino-N^6-methyladenosine (**4b**).

1. Introduction

Diseases due to parasites represent a significant worldwide health problem; as an example, *Plasmodium falciparum* causes the deadliest form of malaria. The disease is endemic in several hundred million people, mainly in developing countries. Remarkably, parasites cannot synthesise purines necessary for many cellular processes, including DNA, RNA and coenzyme synthesis. The purine requirement comes from the host, particularly but not solely via hypoxanthine, from which all other purines required by the parasites are derived. To this end, parasites have purine uptake and salvage pathways, which are sufficiently different from the host to transport, internalise and metabolise the required substrates. The metabolic enzymes along the parasite purine salvage pathway thus are attractive targets for anti-parasitic chemotherapy.[1–3] Purine inhibitors have been designed to specifically block or disrupt the parasite purine uptake or salvage pathway.

Recently, transporters of purine nucleobases and nucleosides into parasites have been intensely studied by using specific inhibitors such as nitrobenzylthioinosine (NBTI) (Fig. 1).[4–8] However the therapeutic application of NBTI has been limited due to its poor pharmacological profile, toxicity and lack of in vivo efficacy. The selective inhibition of such transporters, of which there may be one or several in a particular parasite, still remains a major therapeutic target.[9,10]

Purine analogues have been shown to be potent, versatile small molecule inhibitors and modulators of key biological targets.[11] Furneaux and co-workers developed 5′-methylthioimmucillin-H (MT-ImmH) (Fig. 1) as a selective inhibitor of *P. falciparum* purine nucleoside phosphorylase (PfPNP) with K_d values of 2.7 nM and 303 nM for PfPNP and human-PNP, respectively, showing a selectivity factor of 112.[12] Other purine analogues that demonstrate anti-malarial properties include MDL73811 (Fig. 1) which has an IC_{50} of 2–3 μM against both chloroquine-sensitive and chloroquine-resistant *P. falciparum* strains by inhibiting *S*-adenosylmethionine decarboxylase. More recently, Rodenko et al. have found N^6-diphenylethyl-5′-phenylcarboxamidoadenosine to possess an IC_{50} value of 1.8 μM against chloroquine-resistant *P. falciparum* and similarly the N^6-benzyl-derivative possesses an IC_{50} value of 0.91 μM against *Trypanosoma*. Other purine analogues of interest include 3-deazaguanosine (Fig. 1) which possesses in vitro activity against *Leishmania tropica* with an IC_{50} of 3.6 μM.[13] Hasan et al. also demonstrated that in vivo experiments using the acyclic analogue

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* Corresponding author. Tel.: +44 01223 402222; fax: +44 01223 412178; e-mail: dml@mrc-lmb.cam.ac.uk

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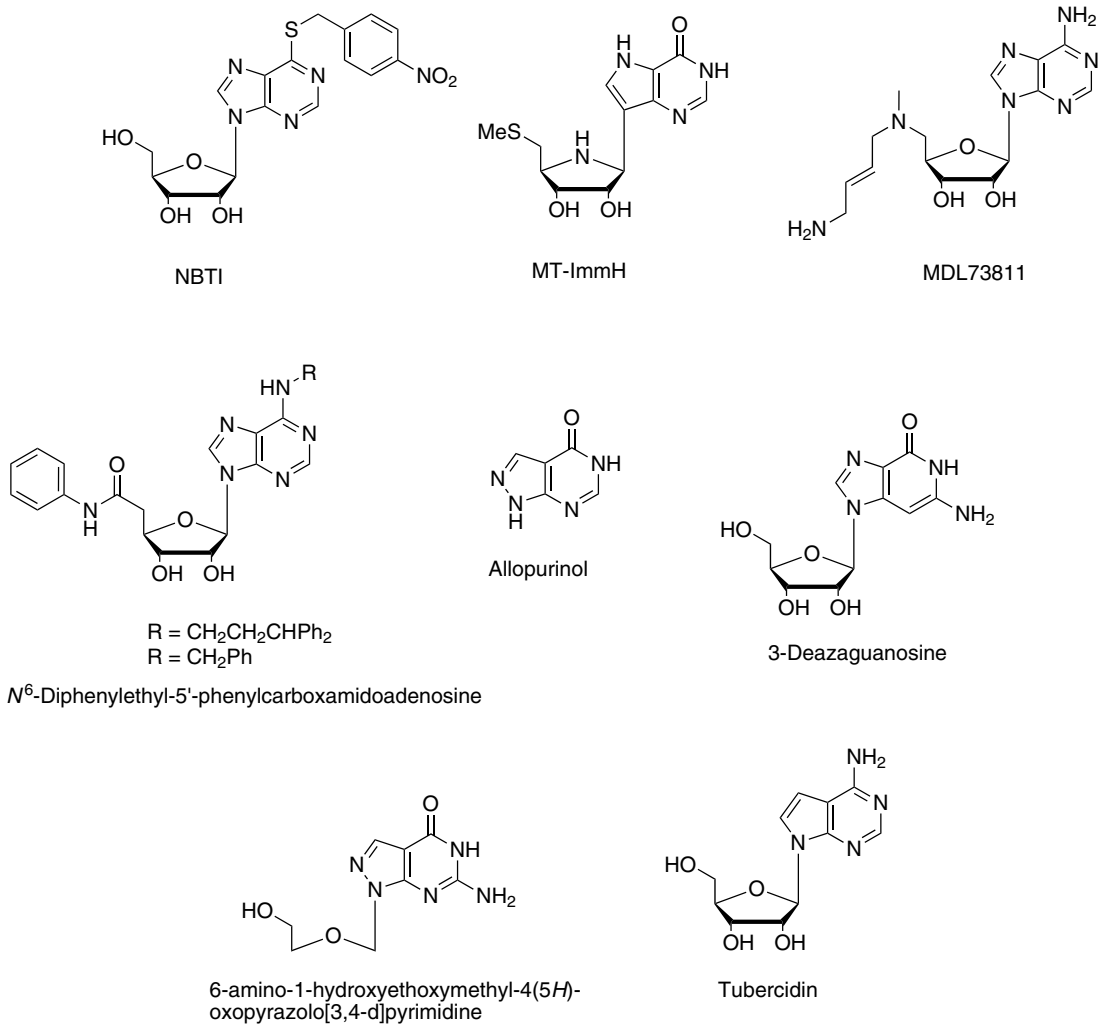


Figure 1. Anti-parasitic agents.

6-amino-1-hydroxyethoxymethyl-4 (5*H*)-oxopyrazolo[3,4-*d*]pyrimidine (Fig. 1) inhibited 94% of amastigotes of *Leishmania donovani* at 50 mg/kg in hamsters.[14] Another example is the adenosine analogue, tubercidin, which was reported to inhibit glycolysis in *Trypanosoma brucei*, though it is toxic.[15] Allopurinol (Fig. 1) is a substrate for Hypoxanthine Guanine Phosphoribosyl Transferase (HGPRT), resulting in the disruption of the synthesis of the parasite's RNA and proteins.[16] As far as we are aware, allopurinol is the only purine analogue that has reached pre-clinical and clinical use against leishmaniasis and trypanosomiasis.[17]

It is well documented that natural nucleobases such as adenine (A) and cytosine (C) have tautomeric constants (K_T) in the order of 10^5 favouring the amino form over the imino form, whereas hypoxanthine, guanine and uracil have K_T values of the same order but favouring the carbonyl over the enol form.[18,19] It was previously shown that, for example, by attaching an electronegative substituent to the exocyclic amino of A or C, the K_T shifts towards the imino form so that the two tautomeric forms, amino and imino, become energetically very similar.[20] Generally, the greater the electronegativity of the substituent on the exocyclic amine, the more favourable is the imino tautomer.[18,19] This work deals with the preparation of a series of analogues that possess functional groups which have both H-bond donor to H-bond acceptor properties in their different tautomeric forms as in N^6-hydroxy adenine (Fig. 2), in the hope that they would be able to present different H-bonding opportunities in the active site of purine transporters or metabolic enzymes along the purine salvage system. Herein, we discuss their synthesis, in vitro and in vivo anti-parasitic activity.

2. Results and discussion

A library of N^6-substituted purines and their corresponding N^9-ribosyl derivatives were synthesised by substituting either 6-chloropurine or 2-amino-6-chloropurine or their corresponding ribonucleosides with *N*-hydroxy and *N*-amino derivatives. Generally, the reactions were carried out at 60 °C for 24 h, in a 1:1 mixture of ethanol and water, to afford the corresponding

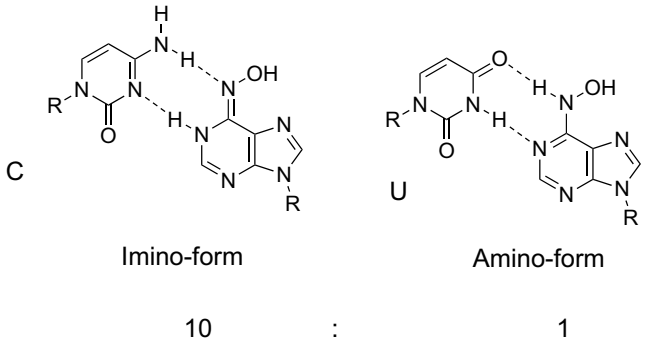


Figure 2. Tautomeric forms of N^6-hydroxyadenine.

nucleobase/ribonucleoside analogues (Table 1), though compounds **4c**, **5c** and **6c** required longer reaction times and higher temperatures. A number of the compounds in this present library have been prepared many years ago but were not fully characterised (Table 1). We have acquired further characterisation data, which is presented in Section 5.

Compound **6c** required a longer reaction time of three days at 80 °C due to the low reactivity of phenylhydrazine as previously observed.[21] Both in the cases of **6a** and **6c**, a 2:1 and 3:2 ratio of N^6-phenyl aminopurine and N^6-amino-N^6-phenylpurine regioisomers were obtained. In order to distinguish between the two regioisomers obtained in the case of **6c**, the compounds were reacted further with acetaldehyde. **6c$_2$** reacted with acetaldehyde to give the corresponding hydrazone **9** in 72% yield, whereas **6c$_1$** did not. It was therefore concluded that **6c$_1$** is N^6-phenylaminoadenine and **6c$_2$** is N^6-amino-N^6-phenyladenine.

In DMSO-d^6 ^{1}H NMR spectra of compounds in the *N*-hydroxy series, **7c** and **8a**, displayed tautomeric ratios of 2:1 and 4:1, respectively, in favour of the imino form, whereas the nucleosides **3a** and **3b** did not display any tautomerism in DMSO-d^6. This is probably due to the rapid exchange of the NH protons on an NMR time scale, making it difficult to observe tautomers. Previously we reported a tautomeric ratio of 9:1 in the case of N^6-methoxy-2,6-diaminopurine 2′-deoxyribonucleoside whilst no tautomerism was observed in the case of N^6-methoxyaminopurine 2′-deoxyribonucleoside in DMSO-d^6.[20]

Based on previous work,[18,20,22] it can be assumed that compounds such as N^6-hydroxyadenine or N^6-hydroxyadenosine also have tautomeric ratios of the order of 10:1 in favour of the imino form (Fig. 2). The imino tautomer of N^6-hydroxyadenine closely resembles inosine and hence may be more likely to be transported into the parasite in this form. The library was thus designed so that we can compare compounds that exist as both tautomers with the corresponding fixed tautomers such as **4a**–**d** and **5a**–**d** (Table 1), which exist in only the amino form due to a lack of exchangeable proton on the N^6-amino group. All the compounds in this library were screened for anti-malarial, anti-leishmanial and anti-trypanosomal activity.

3. Biological activity

The purine analogues were tested against three different strains of *P. falciparum*, namely 3D7 strain which is a standard drug-sensitive laboratory clone of the NF54 isolate, K1 strain (Thailand) which is chloroquine, pyrimethamine and cycloguanil resistant and the VS1 strain (Vietnam) which is highly chloroquine, pyrimethamine and cycloguanil resistant. Several of the analogues were active in the low micromolar range (Table 2) with **4b** possessing the highest potency (IC_{50} 0.29 μM) against the highly multi-drug-resistant VS1 strain. More importantly, **4b** was also non-toxic in KB cells (CC_{50} 1476 μM) giving it a selectivity index (SI) of about 5000. Other interesting compounds in the series include **2b** and **1c** which have SI values of 112 and 267 against the VS1 strain, 136 and 146 against the K1 strain. There was no indication of a correlation between sensitivity to the purine analogues and the resistant phenotypes, clearly showing that the purine analogues could be utilised against multi-drug-resistant strains of *P. falciparum* from endemic areas in which current anti-malarials are already ineffective.

Initial structure–activity relationship (SAR) studies show that when we compare 2-amino-N^6-aminoadenosine (**2b**) and 2-amino-N^6-amino-N^6-methyladenosine (**4b**), addition of a methyl group to the nitrogen at position-6 of purine (Fig. 3) leads to an increase in SI from 112 to 5008 against the VS1 strain, 136 to 410 against the K1 strain and 134 to 533 against the drug-sensitive 3D7 strain. However, the presence of the 2-amino group leads to increased toxicity of the compounds **2a** cf. **2b** (2.3 μM cf. 1364 μM), **2c** cf. **2d** (49.3 μM cf. 1000 μM) and **4a** cf. **4b** (101 μM cf. 1476 μM). Comparing **1c**, **3c** and **7c**, it can be noted that addition of a methyl or phenyl group results in loss of anti-malarial activity. However it is essential to identify the mechanism of action of the analogues before a rational drug design study can be made.

The three compounds that displayed the best in vitro activities and selectivity were administered subcutaneously and orally to infected mice to determine whether the anti-malarial activities were retained in vivo. It was found that after four days treatment at 30 mg/kg (once daily), none of the mice were free from parasites. However, compared to the positive control, in this case pyrimethamine, which effected a 100% inhibition of parasitaemia, **4b**, **2b** and **1c** inhibited parasitaemia by 28%, 14% and 1.8%, respectively (Table 3). Moreover, no overt toxicity was observed over the four days treatment. Upon increasing the dose to 100 mg/kg, it was found that there is no significant change in inhibition of parasitaemia via an oral route of administration for compounds **4b** and **2b** (Table 4). This can be explained by the insolubility of the latter at 100 mg/kg, as the compounds precipitated out of solution overnight. **2b** was found to be the most active when given intravenously at 100 mg/kg, with a 44% inhibition of parasitaemia (Table 4). Post-treatment biopsy showed no overt toxicity.

Table 1. Library of adenosine analogues synthesised from 6-chloropurine/9-ribofuranosylpurine or 2-amino-6-chloropurine/9-ribofuranosylpurine

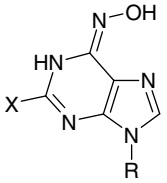
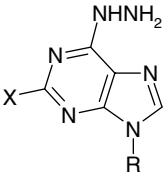
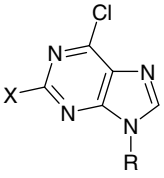
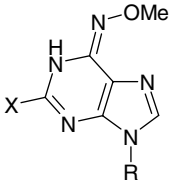
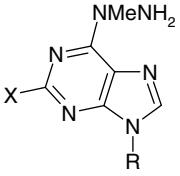
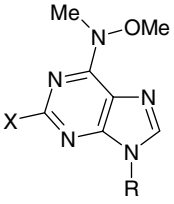
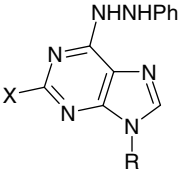
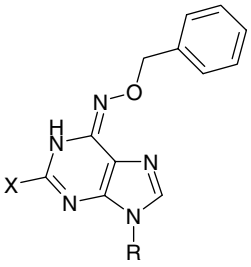
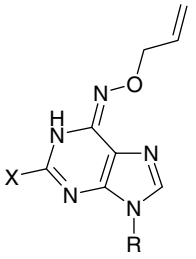
Entry	Starting material	Amine	Product	R	% Yield[a] X = H	% Yield[a] X = NH_2
1		NH_2OH		Ribose	77 **1a**	69 **1b**
				H	87[b] **1c**	78 **1d**
2		NH_2NH_2		Ribose	84 **2a**	90 **2b**
				H	92 **2c**	67 **2d**
3		NH_2OMe		Ribose	55 **3a**	63 **3b**
				H	85 **3c**	90 **3d**
4		$NHMeNH_2$		Ribose	85 **4a**	83 **4b**
				H	87[c] **4c**	95 **4d**
5		NHMeOMe		Ribose	72 **5a**	50 **5b**
				H	86[d] **5c**	n.d.
6		$NHPhNH_2$		Ribose	93 **6a**	n.d.
				H	60[e] **6c**	n.d.
7		NH_2OCH_2Ph		Ribose	46 **7a**	90 **7b**
				H	48 **7c**	n.d.

Table 1 (*continued*)

Entry	Starting material	Amine	Product	R	% Yield[a] X = H	% Yield[a] X = NH_2
8		$NH_2OCH_2CH{=}CH$		Ribose	53 **8a**	n.d.
				H	n.d.	n.d.

n.d., not done.

[a] Chloropurine ribosides were reacted with the corresponding amine at 40–60 °C for 3–24 h. In cases, where hydrochloride salts were used, 1 equiv triethylamine or diisopropylethylamine was used as base.

[b] Reaction was conducted at 60 °C for 30 min.

[c] Reaction mixture was stirred at 90 °C for 12 h.

[d] Reaction mixture was stirred at 116 °C for 4 h.

[e] Reaction mixture was stirred at 80 °C for 3 days.

Table 2. In vitro anti-malarial activity of purine analogues

Compound	IC_{50} (μM)			CC_{50} (μM)	Selectivity index (CC_{50}/IC_{50})		
	3D7	K1	VS1	KB cells	3D7	K1	VS1
1a	11.61	6.90	8.56	44.30	3.8	6.4	5.2
1b	16.03	10.83	13.33	280.7	17.5	25.9	21.1
1c	**7.48**	**10.21**	**5.57**	**1485.8**	**199**	**146**	**267**
1d	27.77	9.03	16.79	45.01	1.6	5.0	2.7
2a	13.26	7.48	7.92	2.26	<1	<1	<1
2b	**10.16**	**10.00**	**12.22**	**1364.00**	**134**	**136**	**112**
2c	23.08	22.08	18.77	49.28	2.1	2.2	2.6
2d	188.95	108.87	89.32	1000.74	5.3	9.2	11.2
3a	11.42	20.57	9.87	6.16	<1	<1	<1
3b	231.94	184.45	224.78	784.16	3.4	4.3	3.5
3c	98.90	353.61	>606	1257.6	12.7	3.6	2.1
3d	428.06	435.35	>555	n.d.	n.d.	n.d.	n.d.
4a	72.71	35.40	22.47	101.00	1.4	2.9	4.5
4b	**2.27**	**3.60**	**0.29**	**1476.0**	**533**	**410**	**5008**
4c	507.89	478.01	418.51	n.d.	n.d.	n.d.	n.d.
4d	>558	>558	>558	n.d.	n.d.	n.d.	n.d.
5a	21.81	12.48	25.22	15.34	<1	1.2	<1
5b	>306	>306	>306	n.d.	n.d.	n.d.	n.d.
5c	>613	>613	>613	n.d.	n.d.	n.d.	n.d.
6a	14.20		10.96	109	7.68		9.9
6c	19.33		19.82	167	8.64		8.43
7a	13.15		14.20	889	67.60		62.6
7b	183.37		126.06	n.d.	n.d.		n.d.
7c	400.48		351.63	n.d.	n.d.		n.d.
8a	33.31		34.00	n.d.	n.d.		n.d.
Chloroquine	0.0024	0.32	0.86	187	77917	584	217
Podophylotoxin	—	—	—	0.048	—	—	—

Tested against three different strains of *Plasmodium falciparum*. The compounds with the highest selectivity indices (1c, 2b and 4b) are highlighted in bold.

3D7: drug-sensitive.

3D7: drug-sensitive.

K1: chloroquine and pyrimethamine resistant.

VS1: highly chloroquine, pyrimethamine and cycloguanil resistant.

n.d., not determined.

The low in vivo efficacy of our compounds might be explained by their high polarity and correspondingly low bioavailability due to their rapid clearance and hence little drug uptake. In order to address this issue, it would be valuable to synthesise prodrugs of the active analogues to make them more lipophilic with improved pharmacokinetics.[23] A second possible explanation is that a different strain of *Plasmodium* was used in the rodent model, implying the purine analogues display different activity against different types of malaria. *P. falciparum* (human malaria) does not grow in or infect rodents so there is a need to use *Plasmodium berghei* to infect the rodents. There are recent reports from some groups using immunodeficient mice that can be infected

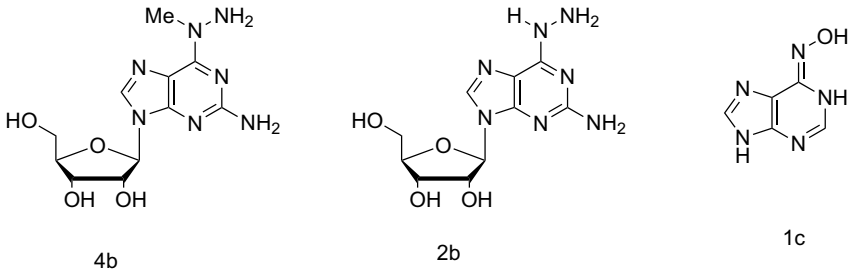


Figure 3. Active anti-malarial compounds.

Table 3. In vivo activity of nucleoside analogues at 30 mg/kg in the *P. berghei* ANKA model (Peters' 4-day test)[23]

Route of administration	Percentage of inhibition of parasitaemia at 30 mg/kg × 4 d (once daily)			
	4b	**2b**	**1c**	Pyrimethamine
sc	28.3	14.0	1.8	100
po	12.3	22.3	3.9	100

sc, subcutaneously.
po, oral.

Table 4. In vivo activity of nucleoside analogues at 100 mg/kg in the *P. berghei* ANKA model (Peters' 4-day test)[23]

Route of administration	Percentage of inhibition of parasitaemia at 100 mg/kg × 4 d (once daily)		
	4b	**2b**	Chloroquine (10 mg/kg)
iv	14.0	43.9[b]	100
po[a]	4.8	26.8	99.7

iv, intravenous.
po, oral.
[a] The compounds **4b** and **2b** precipitated out of solution overnight at 100 mg/kg.
[b] On fourth day of treatment 1 mouse out of the group of 5 was dead, but the remaining four mice looked healthy and post-treatment biopsy did not reveal any overt toxicity.

with *P. falciparum* after pre-treatment with immunosuppressive drugs. However, this model is also controversial.[24] Additionally, rodent malaria strains cannot be maintained in in vitro cultures, although they can be cultured for a short period to do uptake experiments.

There are several hypotheses to try and explain the mechanism of action of the compounds. Currently studies are underway to determine whether the purine analogues are inhibiting either one or more of the metabolic enzymes such as the HGPRT or adenosine deaminase on the purine salvage pathway or whether they are inhibiting the purine transporters PfNT1 or PfENT1.[25] Recently, several groups have identified and characterised *P. falciparum* nucleoside transporters PfNT1 and PfENT1[4–8] which carry nucleobases and nucleosides across the parasite membranes. It has also been shown that deletion of the PfNT1 gene renders the parasite incapable of completing the intraerythrocytic life cycle under standard culture conditions. The purine analogues **4b**, **2b** and **1c** could possibly be inhibiting the PfNT1 or PfENT1 transporters.

Alternatively, the purine analogues could be metabolised to its corresponding monophosphate and triphosphate, which is then incorporated into the parasitic RNA. This has been reported for the purine analogues formycin A and formycin B, which have anti-leishmanial properties.[26] It was revealed that the conversion of formycin A to its corresponding nucleotide and incorporation of the latter in the RNA of the parasite could be potentially harmful to the parasite.[13] Similarly **4b** and **2b** could be phosphorylated to their respective triphosphates by cellular kinasing enzymes and then incorporated into the *P. falciparum* RNA by the parasite's RNA polymerases leading to inhibition of parasite growth via either a chain termination or increased mutagenesis, although the latter explanation would not apply to the fixed tautomer **4b**. Further biochemical evaluation is necessary to confirm the above hypotheses and will be reported subsequently.

Finally the compounds were also assayed against *L. donovani HU3*, *Trypanosoma cruzi tulahuan* and *T. brucei rhodesiense STIB900* using previously described standard methods,[27] but they did not display any significant activity against those parasites.

4. Conclusions

We show here that several N^6-amino-substituted purine analogues from a small library containing both nucleobases and nucleosides display promising anti-malarial activity in vitro and they also reduce parasitaemia in vivo. The three most active compounds were N^6-hydroxyadenine (**1c**), 2-amino-N^6-aminoadenosine (**2b**) and 2-amino-N^6-amino-N^6-methyladenosine (**4b**) with the latter possessing a selectivity index of over 5000 with an IC_{50} 0.29 μM. We have also demonstrated that the active compounds were non-toxic against KB cells in vitro and this was confirmed by the preliminary in vivo experiments. Experiments are currently underway to measure the affinity of these purine analogues for the purine transporters and will be reported independently. Clearly it is important to understand the mechanism(s) of action of the active compounds we have identified to enable more rationally designed analogues for structure–activity studies. Other mechanisms of action, such as the inhibition of the enzymes on the

purine savage pathway, have commenced, but as yet the target enzymes have not been identified, and further studies are underway.

5. Experimental

5.1. Synthetic methods

In general, compounds are synthesised by reaction of an appropriate hydrazine derivative or hydroxylamine derivative with a halogeno-substituted purine or purine nucleoside. Melting points were measured with a Stuart Scientific SMP3 melting point apparatus and are uncorrected. Tandem mass spectra were measured using a API QSTAR Pulsar I tandem mass spectrometer while high resolution mass spectra were recorded on a Bio-Apex II FT-ICR spectrometer. ^{1}H Nuclear Magnetic Resonance (NMR) spectra were recorded at 300 MHz on a Bruker DRX300 instrument whilst ^{13}C NMR spectra were recorded at 125 MHz on a Bruker DRX500 instrument. Unless stated otherwise, deuterated DMSO was used as the NMR solvent with tetramethylsilane (TMS) as the internal standard. Chemical shifts (δ) are given in parts per million (ppm) downfield from TMS. All coupling constants are given in Hertz (Hz). Thin-layer chromatography (tlc) plates with plastic backing coated with Merck Kieselgel PF_{254} were used. Flash chromatography used Merck Kieselgel 60 (230–400 mesh) with an eluent flow rate of ca. 5 mL/min being maintained by air pressure. All commercially available reagents were used as received and where appropriate anhydrous quality material was purchased. All compounds are named according to the IUPAC system and were obtained using the ACD/ILAB web service (http://www.acdlabs.com).

5.2. General procedure for the synthesis of purine analogues

To a solution of 6-chloropurine or 2-amino 6-chloropurine or their corresponding 9-ribofuranosyl derivatives (1.0 equiv) in EtOH/H_2O (1:1), in a sealed tube, nucleophile (10 equiv) was added. The reaction mixture was allowed to stir at 40 °C until reaction was complete (monitoring by TLC). The crude reaction mixture was then evaporated under reduced pressure and then recrystallised in a mixture of dioxane, diethyl ether and methanol. After filtration the white crystals were collected and characterised.

5.2.1. N^6-Hydroxyadenosine (1a).[28,29] Prepared by general procedure from 6-chloro-9-ribofuranosylpurine (0.56 g, 1.94 mmol) and hydroxylamine (50% solution in water) (1.2 mL, 19.4 mmol) in EtOH/H_2O (1:1) (10 mL) to give product (0.43 g, 77%) as a white solid; δ_C 157.0, 148.6, 146.3, 139.2, 124.9, 87.9, 86.1, 74.2, 70.9, 62.0; *m/z* (HRMS). Found: $(M+H)^+$, 284.0995, $C_{10}H_{13}N_5O_5$ requires $(M+H)^+$ 284.0995, deviation 0.0 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 265 (11900); λ_{min} 231; pH 1: λ_{max} 265 (17700); λ_{min} 232; pH 12: λ_{max} 295 (10100); λ_{min} 241; ε260 (M) 12300.

5.2.2. 2-Amino-N^6-hydroxyadenosine (1b).[30] Prepared by general procedure from 2-amino-6-chloro-9-ribofuranosylpurine (0.64 g, 2.11 mmol) and hydroxylamine (1.3 mL, 21.1 mmol) in EtOH/H_2O (1:1) (10 mL) to give product (0.43 g, 69%) as a white solid; δ_H 10.00 (1 H, br s, NH), 7.98 (1H, s, H8), 6.82 (1H, br s, OH), 5.67 (1H, d, *J* 5.9, 1′-H), 5.41 (1H, d, *J* 6.1, 2′-OH), 5.14 (1H, br s, 3′-OH), 4.39 (1H, app t, *J* 5.4, 2′-H), 4.07 (1H, app t, *J* 4.1, 3′-H), 3.87 (1H, dd, *J* 7.3, 3.7, 4′-H), 3.60 (1H, dd, *J* 12.0, 3.9, 5′-H_a), 3.50 (1H, dd, *J* 12.0, 3.9, 5′-H_b), 3.34 (2H, br s, NH_2); δ_C 163.0, 153.5, 148.0, 136.4, 110.7, 87.1, 85.7, 74.1, 70.8, 61.8; *m/z* (HRMS). Found: $(M+H)^+$, 297.1103, $C_{10}H_{14}N_6O_5$ requires $(M+H)^+$ 299.1104, deviation −0.3 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 281 (10900); λ_{min} 242; pH 1: λ_{max} 294 (10900), 257 (11200); λ_{min} 274, 237; pH 12: λ_{max} 296 (10800); λ_{min} 261; ε260 (M) 9200.

5.2.3. N^6-Hydroxyadenine (1c).[31] Prepared by general procedure from 6-chloropurine (0.25 g, 1.61 mmol) and hydroxylamine (50% in water) (3 mL) in water (5 mL) heated at 60 °C for 0.5 h to give product (0.168 g, 87%) as a white solid; δ_H 12.45 (1 H, br s, OH), 10.91 (1H, br s, NH), 9.44 (1H, br s, NH), 8.09 (1H, s, H8), 7.96 (1H, s, H2). UV: λ_{max} (nm) (10% MeOH in H_2O) 267 (15200); λ_{min} 231; pH 1: λ_{max} 271 (19500); λ_{min} 235; pH 12: λ_{max} 265 (8040); λ_{min} 248; ε260 (M) 14400.

5.2.4. 2-Amino-N^6-hydroxyadenine (1d). Prepared by general procedure from 2-amino-6-chloropurine (0.50 g, 2.98 mmol) and hydroxylamine (50% in water) (0.91 mL, 29.8 mmol) in EtOH/H_2O (1:1) (10 mL) heated at 60 °C for 24 h to give product (0.39 g, 78%) as a white solid; mp >300 °C; δ_H 9.54 (1H, s, NH), 7.65 (1H, s, H8), 6.41 (2H, s, NH_2); δ_C 164, 153.9, 150.3, 144.8, 138.1, 116.1; *m/z* (HRMS). Found: $(M+H)^+$, 167.0687, $C_5H_6N_6O$ requires $(M+H)^+$ 167.0681, deviation 3.2 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 282 (7500), 251 (6100); λ_{min} 261, 240; pH 1: λ_{max} 282 (8400), 251 (8400); λ_{min} 266, 238; pH 12: λ_{max} 274 (2950); ε260 (M) 5700.

5.2.5. N^6-Aminoadenosine (2a).[32,33] Prepared by general procedure from 6-chloro-9-ribofuranosylpurine (0.69 g, 2.41 mmol) and hydrazine monohydrate (1.17 mL, 24.1 mmol) in EtOH/H_2O (1:1) (10 mL) to give product (0.57 g, 84%) as a white solid; δ_H 9.03 (1H, br s, NH), 8.34 (1H, s, H8), 8.23 (1H, s, H2), 5.88 (1H, d, *J* 6.2, 1′-H), 5.45 (1H, d, *J* 6.3, 2′-OH), 5.40 (1H, dd, *J* 7.0, 2.3, 5′-OH), 5.20 (1H, d, *J* 4.6, 3′-OH), 4.58 (1H, app t, *J* 5.4, 2′-H), 4.13 (1H, dd, *J* 7.8, 4.6, 3′-H), 3.95 (1H, dd, *J* 6.4, 3.3, 4′-H), 3.66 (1H, m, 2, 5′-H_a), 3.53 (1H, m, 5′-H_b), 3.34 (2H, s, NH_2); δ_C 155.9, 152.7, 148.7, 140.1, 118.9, 88.3, 86.3, 73.9, 71.0, 62.0; *m/z* (HRMS). Found: $(M+H)^+$, 283.1154, $C_{10}H_{15}N_6O_4$ requires $(M+H)^+$ 283.1155, deviation −0.3 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 266 (13600); λ_{min} 231; pH 1: λ_{max} 261 (14300); λ_{min} 231; pH 12: λ_{max} 266 (14200), λ_{min} 234; ε260 (M) 12600.

5.2.6. 2-Amino-N^6-amino-adenosine (2b).[30] Prepared by general procedure from 2-amino-6-chloro-9-ribofurano-

sylpurine (0.56 g, 1.85 mmol) and hydrazine monohydrate (0.90 mL, 16.6 mmol) in EtOH/H_2O (1:1) (10 mL) to give product (0.49 g, 90%) as a white solid; δ_H 8.55 (1H, br s, OH), 7.93 (1H, s, H8), 5.90 (2H, s, NH_2), 5.75 (1H, d, *J* 6.3, 1′-H), 5.43 (1H, dd, *J* 6.6, 4.9, 5′-OH), 5.39 (1H, d, *J* 6.3, 2′-OH), 5.13 (1H, d, *J* 4.5, 3′-OH), 4.52 (1H, dd, *J* 11.3, 6.1, 2′-H), 4.46 (2H, br s, NH_2), 4.11 (1H, dd, *J* 7.7, 4.6, 3′-H), 3.92 (1H, dd, *J* 6.7, 3.5, 4′-H), 3.66 (1H, dt, *J* 12.2, 4.2, 5′-H_a), 3.54 (1H, ddd, *J* 12.2, 6.6, 4.0, 5′-H_b); δ_C 160.3, 156.3, 151.4, 136.3, 112.9, 87.3, 85.9, 73.7, 71.1, 62.1; *m/z* (HRMS). Found: $(M+H)^+$, 298.1259, $C_{10}H_{15}N_7O_4$ requires $(M+H)^+$ 298.1264, deviation −1.7 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 282 (12300), 260 (9200); λ_{min} 265, 242; pH 1: λ_{max} 290 (10700), 254 (9600); λ_{min} 271, 236; pH 12: λ_{max} 282 (11600); λ_{min} 243; ε260 (M) 9200.

5.2.7. N^6-Aminoadenine (2c).[34,35] δ_H 12.77 (1H, br s, NH), 8.69 (1H, br s, NH), 8.18 (1H, s, H8), 8.09 (1H, s, H2), 4.60 (2H, s, NH_2). UV: λ_{max} (nm) (10% MeOH in H_2O) 268 (21600); λ_{min} 234; pH 1: λ_{max} 267 (23100); λ_{min} 233; pH 12: λ_{max} 272 (24900), λ_{min} 240; ε260 (M) 18400.

5.2.8. 2-Amino-N^6-aminoadenine (2d).[34] δ_H 12.06 (1H, br s, NH), 8.22 (1H, s, NH), 7.73 (1H, s, H8), 5.73 (2H, s, NH_2). UV: λ_{max} (nm) (10% MeOH in H_2O) 283 (5080), λ_{min} 261; pH 1: λ_{max} 286 (10800); λ_{min} 258; pH 12: λ_{max} 287 (10300); λ_{min} 258; ε260 (M) 2960.

5.2.9. N^6-Methoxyadenosine (3a).[28,36,37] Prepared by general procedure from 6-chloro-9-ribofuranosylpurine (0.57 g, 2.00 mmol) and methoxylamine (0.93 g, 20.0 mmol) in EtOH/H_2O (1:1) (10 mL) to give product (0.33 g, 55%) as a white solid; δ_H (DMSO, D_2O wash) 8.21 (1H, s, H8), 7.76 (1H, s, H2), 5.78 (1H, d, *J* 5.8, 1′-H), 4.45 (1H, app t, *J* 5.4, 2′-H), 4.09 (1H, app t, *J* 4.2, 3′-H), 3.91 (1H, app q, *J* 3.6, 4′-H), 3.75 (3H, s, OMe), 3.62 (1H, dd, *J* 12.0, 3.8, 5′-H_a), 3.51 (1H, dd, *J* 12.0, 3.9, 5′-H_b); δ_C (DMSO) 164.6, 153.4, 146.4, 138.6, 118.5, 87.9, 86.0, 74.4, 70.7, 62.1, 61.8; *m/z* (HRMS). Found: $(M+H)^+$, 298.1171, $C_{11}H_{15}N_5O_5$ requires $(M+H)^+$ 298.1165, deviation 2.1 ppm; UV: λ_{max} (nm) (10% MeOH in H_2O) 268 (13200); λ_{min} 236; pH 1: λ_{max} 267 (18300); λ_{min} 235; pH 12: λ_{max} 280 (14700); λ_{min} 241; ε260 (M) 11700.

5.2.10. 2-Amino-N^6-methoxyadenosine (3b).[38,39] Prepared by general procedure from 2-amino-6-chloro-9-ribofuranosylpurine (0.52 g, 1.71 mmol) and methoxylamine (0.80 g, 17.1 mmol) in EtOH/H_2O (1:1) (10 mL) to give product (0.34 g, 63%) as a white solid; δ_H 8.31 (1H, s, H8), 7.04 (2H, s, NH_2), 5.70 (1H, d, *J* 5.2, 1′-H), 5.40 (3H, br s, 3× OH), 4.37 (1H, app t, *J* 5.0, 2′-H), 4.09 (1H, app t, *J* 4.4, 3′-H), 3.89 (1H, dd, *J* 7.3, 3.6, 4′-H), 3.78 (3H, s, OMe), 3.63 (1H, dd, *J* 12.0, 3.6, 5′-H_a), 3.53 (1H, dd, *J* 12.0, 3.7, 5′-H_b); δ_C 164.7, 153.3, 147.0, 135.7, 108.3, 87.5, 85.7, 74.3, 70.4, 62.7, 61.4; *m/z* (HRMS). Found: $(M+H)^+$, 313.1266, $C_{11}H_{16}N_6O_5$ requires $(M+H)^+$ 313.1260, deviation 1.8 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 280 (14800); λ_{min} 242; pH 1: λ_{max} 297 (13100), 256 (10900); λ_{min} 273, 239; pH 12: λ_{max} 288 (19200); λ_{min} 253; ε260 (M) 10500.

5.2.11. N^6-Methoxyadenine (3c).[28] Prepared by general procedure from 6-chloropurine (1.00 g, 6.47 mmol) and methoxylamine (6.00 g, 127 mmol) in 1-butanol (100 mL) heated at 70–80 °C for 12 h to give product (0.91 g, 85%) as a pale yellow solid; δ_H 8.11 (1H, s, H8), 7.75 (1H, s, H2), 3.77 (3H, s, OMe); UV: λ_{max} (nm) (10% MeOH in H_2O) 270 (9900); λ_{min} 234; pH 1: λ_{max} 274 (9800); λ_{min} 238; pH 12: λ_{max} 276 (9900), λ_{min} 241; ε260 (M) 8200.

5.2.12. 2-Amino-N^6-Methoxyadenine (3d). Prepared by general procedure from 2-amino-6-chloropurine (0.47 g, 2.79 mmol) and methoxylamine (1.31 g, 27.9 mmol) in EtOH/H_2O (1:1) (10 mL) heated at 60 °C for 24 h to give product (0.45 g, 90%) as a pale greyish solid; mp >300 °C; δ_H 8.25 (1H, s, H8), 7.31 (2H, s, NH_2), 3.80 (3H, s, OMe); δ_C 153.5, 150.4, 138.8, 117.8, 63.4; *m/z* (HRMS). Found: $(M+Na)^+$, 203.0664, $C_6H_8N_6O$ requires $(M+Na)^+$ 203.0657, deviation 3.4 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 282 (9400); λ_{min} 240; pH 1: λ_{max} 283 (9100); λ_{min} 240; pH 12: λ_{max} 291 (9050); λ_{min} 250; ε260 (M) 7300.

5.2.13. N^6-Amino-N^6-methyladenosine (4a).[28] Prepared by general procedure from 6-chloro-9-ribofuranosylpurine (0.53 g, 1.85 mmol) and *N*-methylhydrazine (1.0 mL, 18.5 mmol) in EtOH/H_2O (1:1) (10 mL) to give product (0.46 g, 85%) as a white solid; δ_H 8.37 (1H, s, H8), 8.19 (1H, s, H2), 5.89 (1H, d, *J* 6.0, 1′-H), 5.56 (2H, br s, NH_2), 5.46 (1H, d, *J* 6.2, 2′-OH), 5.38 (1H, dd, *J* 6.8, 4.7, 5′-OH), 5.20 (1H, d, *J* 4.7, 3′-OH), 4.57 (1H, dd, *J* 11.2, 5.9, 2′-H), 4.13 (1H, dd, *J* 8.0, 4.6, 3′-H), 3.95 (1H, m, 4′-H), 3.70–3.50 (5H, m, 5′-H_a, 5′-H_b, NMe); δ_C 153.9, 152.2, 149.9, 139.0, 119.0, 88.3, 86.2, 74.0, 71.0, 62.0, 37.5; *m/z* (HRMS). Found: $(M+H)^+$, 297.1315, $C_{11}H_{16}N_6O_4$ requires $(M+H)^+$ 297.1311, deviation 1.3 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 275 (14300); λ_{min} 236; pH 1: λ_{max} 267 (15500); λ_{min} 234; pH 12: λ_{max} 276 (13000); λ_{min} 238; ε260 (M) 9500.

5.2.14. 2-Amino-N^6-amino-N^6-methyladenosine (4b).[30] Prepared by general procedure from 2-amino-6-chloro-9-ribofuranosylpurine (0.50 g, 1.66 mmol) and *N*-methylhydrazine (0.89 mL, 16.6 mmol) in EtOH/H_2O (1:1) (10 mL) to give product (0.43 g, 83%) as a white solid; δ_H 7.95 (1H, s, H8), 5.88 (2H, br s, NH_2), 5.74 (1H, d, *J* 6.1, 1′-H), 5.39 - 5.33 (2H, m, 2′-OH, 5′-OH), 5.12 (1H, d, *J* 4.6, 3′-OH), 4.46 (1H, dd, *J* 11.4, 5.9, 2′-H), 4.08 (1H, m, 3′-H), 3.88 (1H, m, 4′-H), 3.62 (1H, dt, *J* 12.0, 4.2, 5′-H_a), 3.55–3.41 (4H, m, 5′-H_b and NMe); δ_C 159.7, 154.4, 152.6, 135.5, 113.2, 87.2, 85.8, 73.6, 71.1, 62.0, 49.0; *m/z* (HRMS). Found: $(M+H)^+$, 312.1422, $C_{11}H_{17}N_7O_4$ requires $(M+H)^+$ 312.1420, deviation 0.6 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 288 (12100); λ_{min} 250; pH 1: λ_{max} 297 (12200), 256 (11200); λ_{min} 274, 237; pH 12: λ_{max} 287 (13400); λ_{min} 251; ε260 (M) 8500.

5.2.15. N^6-Amino-N^6-methyladenine (4c).[28] Prepared by general procedure from 6-chloropurine (0.20 g, 1.29 mmol) and *N*-methylhydrazine (1 mL) in water (5 mL) heated at 90 °C for 12 h to give product (0.213 g, 87%) as a white solid; δ_H 8.18 (1H, s, H8), 8.09 (1H, s, H2), 3.41 (3H, s, NMe). UV: λ_{max} (nm) (10% MeOH in H_2O) 279 (15200); λ_{min} 236; pH 1: λ_{max} 281 (16200); λ_{min} 239; pH 12: λ_{max} 281 (11300); λ_{min} 245; ε260 (M) 8420.

5.2.16. 2-Amino-N^6-amino-N^6-methyladenine (4d).[28] δ_H 7.88 (1H, s, H8), 6.67 (2H, s, NH_2), 3.41 (3H, s, NMe). UV: λ_{max} (nm) (10% MeOH in H_2O) 289 (11700); λ_{min} 264; pH 1: λ_{max} 281 (14000), 253 (12300); λ_{min} 265, 240; pH 12: λ_{max} 293 (12400); λ_{min} 263; ε260 (M) 6350.

5.2.17. N^6-Methoxy-N^6-methyladenosine (5a).[40] Prepared by general procedure from 6-chloro-9-ribofuranosylpurine (0.80 g, 2.82 mmol) and *N*, *O*-dimethylhydroxylamine (1.75 g, 28.2 mmol) in EtOH/H_2O (1:1) (10 mL) heated at 60 °C for 24 h to give product (0.63 g, 72%) as a white solid; δ_H 8.85 (1H, s, OH), 8.61 (1H, s, H8), 8.51 (1H, s, H2), 5.93 (1H, d, *J* 7.0, 1′-H), 5.46 (1H, s, OH), 5.20 (1H, s, OH), 4.56 (1H, m, 2′-H), 4.14 (1H, m, 3′-H), 3.95 (1H, m, 4′-H), 3.85 (3H, S, OMe), 3.68–3.33 (5H, m, NMe, 5′-H_a and 5′-H_b); δ_C (D_2O) 153.5, 151.4, 149.3, 140.3, 118.9, 88.5, 85.8, 74.0, 70.8, 61.7, 61.1, 34.8; *m/z* (HRMS). Found: $(M+H)^+$, 312.1316, $C_{12}H_{17}N_5O_5$ requires $(M+H)^+$ 312.1308, deviation 2.7 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 275 (7600); λ_{min} 235; pH 1: λ_{max} 269 (12400); λ_{min} 235; pH 12: λ_{max} 275 (13000); λ_{min} 238; ε260 (M) 4900.

5.2.18. 2-Amino-N^6-methoxy-N^6-methyladenosine (5b). Prepared by general procedure from 2-amino-6-chloro-9-ribofuranosylpurine (0.30 g, 0.98 mmol) and *N*,*O*-dimethylhydroxylamine (0.60 g, 9.8 mmol) in EtOH/H_2O (1:1) (10 mL) heated at 60 °C for 24 h to give product (0.16 g, 50%) as a white solid; mp 120–122 °C; δ_H (DMSO, D_2O wash) 8.49 (1H, s, H8), 5.78 (1H, d, *J* 5.3, 1′-H), 4.40 (1H, app t, *J* 5.1, 2′-H), 4.12 (1H, app t, *J* 4.4, 3′-H), 3.92 (1H, m, 4′-H), 3.87 (3H, s, OMe), 3.80 (3H, s, NMe), 3.65 (1H, dd, *J* 12.0 and 3.8, 5′-H_a), 3.54 (1H, dd, *J* 12.0 and 3.7, 5′-H_b); δ_C (D_2O) 156.3, 151.4, 149.0, 140.3, 119.4, 88.4, 85.7, 74.1, 70.5, 61.7, 61.4, 37.3; *m/z* (HRMS). Found: $(M+H)^+$, 327.1418, $C_{12}H_{18}N_6O_5$ requires $(M+H)^+$ 327.1417, deviation 0.2 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 290 (11500), 255 (8600); λ_{min} 270, 248; pH 1: λ_{max} 302 (12350), 256 (10800); λ_{min} 274, 241; pH 12: λ_{max} 290 (11600), 254 (7900); λ_{min} 269, 248; ε260 (M) 8250.

5.2.19. N^6-Methoxy-N^6-methyladenine (5c).[41] Prepared by general procedure from 6-chloropurine (1.08 g, 7.0 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (2.73 g, 28.0 mmol) with triethylamine (3.54 g, 35 mmol) in 1-butanol (14 mL) heated under reflux for 4 h to give product (1.07 g, 86%) as a pale yellow solid; UV: λ_{max} (nm) (10% MeOH in H_2O) 279 (5000); λ_{min} 235; pH 1: λ_{max} 284 (5800); λ_{min} 241; pH 12: λ_{max} 280 (4600); λ_{min} 245; ε260 (M) 2900.

5.2.20. N^6-Phenylamino-adenosine ($6a_1$). Prepared by general procedure from 6-chloro-9-ribofuranosylpurine (0.50 g, 1.73 mmol) and phenylhydrazine (1.7 mL, 17.3 mmol) in EtOH/H_2O (1:1) (10 mL), heated at 60 °C for 24 h, to give a mixture of regioisomers **$6a_1$** and **$6a_2$** (0.39 g, 63%), which was separated by flash column chromatography eluting with DCM/10% MeOH to afford pure **$6a_1$** (R_f 0.35 in DCM/20% MeOH) as a yellow solid and **$6a_2$** (R_f 0.29 in DCM/20% MeOH) as mixture together with **$6a_1$**.

5.2.21. N^6-Phenylamino-adenosine ($6a_1$). Mp 200–202 °C; δ_H 9.80 (1H, br s, NH), 8.46 (1H, s, H8), 8.25 (1H, s, H2), 8.04 (1H, br s, NH), 7.14 (2H, dd, *J* 8.3, 7.4, phenyl-H), 6.77 (2H, d, *J* 7.6, phenyl-H), 6.70 (1H, t, *J* 7.3, phenyl-H), 5.95 (1H, d, *J* 5.9, 1′-H), 5.51 (1H, d, *J* 6.2, 2′-OH), 5.36 (1H, t, *J* 5.6, 5′-OH), 5.24 (1H, d, *J* 4.6, 3′-OH), 4.66 (1H, dd, *J* 11.2, 5.9, 2′-H), 4.19 (1H, app dd, *J* 7.7, 4.6, 3′-H), 4.00 (1H, app dd, *J* 6.6, 3.4, 4′-H), 3.71 and 3.59 (2× 1H, 2× m, 5′-H_a and 5′-H_b); δ_C 153.1, 150.5, 142.5, 140.3, 139.3, 129.6, 119.1, 112.9, 112.7, 88.7, 86.7, 74.3, 71.5, 62.5; *m/z* (HRMS). Found: $(M+H)^+$, 359.1465, $C_{16}H_{18}N_6O_4$ requires $(M+H)^+$ 359.1468, deviation −0.8 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 266 (7900); λ_{min} 242; pH 1: λ_{max} 263 (9300); λ_{min} 239; pH 12: λ_{max} 340 (7600); λ_{min} 257; ε260 (M) 7500.

5.2.22. N^6-Phenylamino adenine ($6c_1$) and N^6-amino-N^6-phenyladenine ($6c_2$). Prepared by general procedure from 6-chloropurine (0.52 g, 3.41 mmol) and phenylhydrazine (3.4 mL) in EtOH/H_2O 1:1 (10 mL) heated at 80 °C for 3 days to give a 1.5:1 ratio of regioisomers **$6c_1$** and **$6c_2$** (0.46 g, 60%), which was separated by flash column chromatography eluting with DCM/10% MeOH to afford **$6c_1$** (Rf 0.65 in DCM/20% MeOH) as an off-white solid and **$6c_2$** (Rf 0.37 in DCM/20% MeOH) as pale pink solid, respectively.

5.2.23. N^6-Phenylaminoadenine ($6c_1$). Mp 245–248 °C; δ_H (CD_3OD) 8.28 (1H, s, H8), 8.13 (1H, s, H2), 7.17 (2H, dd, *J* 8.4, 7.4, ArH), 6.91 (2H, d, *J* 7.7, ArH), 6.82 (1H, t, *J* 7.3, ArH); δ_C (CD_3OD) 151.6, 151.2, 148.8, 144.8, 142.0, 128.3, 119.8, 119.4, 112.7; *m/z* (MS/MS by ESI) 227 (M+H), 210 (M-NH_2), 136 (M-NPh+H), 92 (C_6H_5N+H); *m/z* (HRMS). Found: $(M+H)^+$, 227.1046, $C_{11}H_{10}N_6$ requires $(M+H)^+$ 227.1040, deviation −2.9 ppm; UV: λ_{max} (nm) (10% MeOH in H_2O) 271 (5200); λ_{min} 245; pH 1: λ_{max} 270 (5200); λ_{min} 241; pH 12: λ_{max} 274 (5800); λ_{min} 250; ε260 (M) 4330.

5.2.24. N^6-Amino-N^6-phenyladenine ($6c_2$). Mp 238–241 °C; δ_H (CD_3OD) 8.27 (1H, s, H8), 8.19 (1H, s, H2), 7.61 (2H, d, *J* 8.3, ArH), 7.50 (2H, t, *J* 7.9, ArH), 7.33 (1H, t, *J* 7.4, ArH); δ_C (CD_3OD) 151.2, 150.0, 145.5, 144.8, 142.4, 128.0, 126.3, 124.5, 112.8; *m/z* (MS/MS by ESI) 227 (M+H), 210 (M−NH_2), 92 (C_6H_5N+H), 77 (Ph); *m/z* (HRMS). Found: $(M+H)^+$, 227.1046, $C_{11}H_{10}N_6$ requires $(M+H)^+$ 227.1040, deviation −2.7 ppm; UV: λ_{max} (nm) (10% MeOH in H_2O) 287 (6280); λ_{min} 247; pH 1: λ_{max} 291 (7230); λ_{min} 247; pH 12: λ_{max} 293 (6600); λ_{min} 256; ε260 (M) 2850.

5.3. 6-(2-Ethylidene-1-phenylhydrazinyl)-9H-purine (9)

To a solution of N^6-amino-N^6-phenyladenine (**6c$_2$**, 243 mg, 1.07 mmol) in DMF, acetaldehyde (72 μL, 1.29 mmol) was added. After stirring at room temperature for 2 h, the organic solvent was evaporated under reduced pressure. The resulting crude product was purified by flash column chromatography eluting with 5% MeOH/DCM to give product (195 mg, 72%) as a pale yellow solid; mp 163–165 °C; δ_H 8.43 (1H, s, H8), 8.23 (1H, s, H2), 7.65 (2H, t, *J* 7.4, 2× ArH), 7.56 (1H, t, *J* 7.3, ArH), 7.32 (2H, d, *J* 7.3, 2× ArH), 6.80 (1H, q, *J* 5.4, $CH_3\underline{C}$=N), 2.11 (3H, d, *J* 5.4, CH_3); δ_C (CD_3OD) 158.6, 151.0, 150.8, 143.8, 143.6, 135.5, 130.1, 129.9, 129.4, 128.9, 16.5; *m*/*z* (HRMS). Found: $(M+H)^+$, 253.1188, $C_{13}H_{12}N_6$ requires $(M+H)^+$ 253.1196, deviation −3.2 ppm. UV λ_{max} (nm) (H_2O) 294 (16720), λ_{min} 244; pH 1: λ_{max} 304 (23570); λ_{min} 252; pH 12: λ_{max} 297 (20370); λ_{min} 254.

5.3.1. N^6-Benzyloxyadenosine (7a). Prepared by general procedure from 6-chloro-9-ribofuranosylpurine (0.43 g, 1.5 mmol) and *O*-benzylhydroxylamine hydrochloride (1.0 g, 6.3 mmol) in EtOH/H_2O (1:1) (10 mL) and triethylamine (1.5 mL, 10.5 mmol), heated at 60 °C for 24 h, to give product (0.26 g, 46%) as a white solid; δ_H (MeOD) 8.35 (1H, br s, NH), 8.01 (1H, s, H8), 7.59 (1H, s, H2), 7.44–7.41 (2H, m, 2× ArH), 7.35–7.26 (3H, m, 3× ArH), 5.85 (1H, d, *J* 6.0, 1′-H), 5.07 (2H, s, OCH_2), 4.57 (1H, app t, *J* 5.5, 2′-H), 4.27 (1H, m, 3′-H), 4.11 (1H, m, 4′-H), 3.84 (1H, dd, *J* 12.4, 2.2, 5′-H_a), 3.71 (1H, dd. *J* 12.4, 2.5, 5′-H_b); δ_C 164.0, 151.9, 148.8, 141.9, 137.3, 129.2, 129.1 128.9, 128.6, 128.5, 118.9, 88.2, 86.4, 75.5, 74.9, 71.2, 62.3; *m*/*z* (HRMS). Found: $(M+Na)^+$, 396.1276, $C_{17}H_{19}N_5O_5Na$ requires $(M+Na)^+$ 312.1284, deviation −1.9 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 268 (12400); λ_{min} 235; pH 1: λ_{max} 268 (14600); λ_{min} 236; pH 12: λ_{max} 285 (12000); λ_{min} 236; ε260 (M) 10700.

5.3.2. 2-Amino-N^6-benzyloxyadenosine (7b). Prepared by general procedure from 2-amino-6-chloro-9-ribofuranosylpurine (0.59 g, 1.95 mmol) and *O*-benzylhydroxylamine hydrochloride (1.86 g, 11.7 mmol) in EtOH/H_2O (1:1) (10 mL) and diisopropylethylamine (2.71 mL, 15.6 mmol), heated at 60 °C for 24 h, to give product (0.68 g, 90%) as a white solid; mp 109–111 °C; δ_H (D_2O) 7.74 (1H, s, H8), 7.46 (2H, d, *J* 7.4, 2× ArH), 7.36 (2H, t, *J* 7.4, 2× ArH), 7.30 (1H, t, *J* 7.2, benzyl-H), 5.77 (1H, d, *J* 6.2, 1′-H), 5.06 (2H, s, OCH_2), 4.66 (1H, app t, *J* 5.5, 2′-H), 4.30 (1H, dd, *J* 5.0, 2.5, 3′-H), 4.13 (1H, m, 4′-H), 3.87 (1H, dd, *J* 12.4, 2.4, 5′-H_a), 3.73 (1H, m, 5′-H_b); δ_C (CD_3OD) 164.0, 153.9, 148.8, 141.9, 137.3, 128.8, 128.4, 128.1, 127.9, 127.5, 114.0, 89.7, 85.5, 74.4, 70.6, 62.6, 55.0; *m*/*z* (HRMS). Found: $(M+H)^+$, 389.1574, $C_{17}H_{21}N_6O_5$ requires $(M+H)^+$ 389.1573, deviation 0.1 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 280 (11800); λ_{min} 242; pH 1: λ_{max} 297 (9000), 257 (7000); λ_{min} 273, 241; pH 12: λ_{max} 290 (10100); λ_{min} 253; ε260 (M) 8200.

5.3.3. N^6-Benzyloxyadenine (7c). Prepared by general procedure from 6-chloropurine (0.99 g, 6.41 mmol) and *O*-benzylhydroxylamine hydrochloride (5.57 g, 34.9 mmol) in EtOH/H_2O (1:1) (20 mL) and diisopropylethylamine (6.0 mL, 34.6 mmol), heated at 60 °C for 24 h, to give the desired product (0.75 g, 48%) as a white solid; mp 134–136 °C. *m*/*z* (HRMS). Found: $(M+Na)^+$, 264.0858, $C_{12}H_{11}N_5ONa$ requires $(M+Na)^+$ 264.0861, deviation −1.3 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 272 (4700); λ_{min} 236; pH 1: λ_{max} 277 (4400); λ_{min} 242; pH 12: λ_{max} 277 (10100); λ_{min} 241; ε260 (M) 3700. δ_C (CD_3OD) 164.0, 151.9, 150.3, 144.8, 137.3, 127.5 (3× C), 127.0 (2× C), 117.8, 75.3. ^{1}H NMR showed a 2:1 ratio of imino-amino tautomers.

Major tautomer. δ_H 13.02 (1H, s, NH), 11.23 (1H, s, NH), 7.85 (1H, s, H8), 7.57 (1H, s, H2), 7.44–7.28 (5H, m, 5× ArH), 5.06 (2H, s, OCH_2).

Minor tautomer. δ_H 12.85 (1H, s, NH), 11.13 (1H, s, NH), 7.77 (1H, s, H8), 7.57 (1H, s, H2), 7.44–7.28 (5H, m, 5× ArH), 5.03 (2H, s, OCH_2).

5.3.4. N^6-Allyloxyadenosine (8a). Prepared by general procedure from 6-chloro-9-ribofuranosylpurine (1.13 g, 3.95 mmol) and *O*-allylhydroxylamine hydrochloride (2.60 g, 23.7 mmol) in EtOH (10 mL) and diisopropylethylamine (5.5 mL, 31.6 mmol), heated at 60 °C for 24 h, to give the desired product (0.67 g, 53%) as a white solid; mp 147–149 °C; *m*/*z* (HRMS). Found: $(M+H)^+$, 324.1307, $C_{13}H_{18}N_5O_5$ requires $(M+H)^+$ 324.1308, deviation −0.4 ppm. UV: λ_{max} (nm) (10% MeOH in H_2O) 268 (ε = 13800); λ_{min} 235; pH 1: λ_{max} 268 (17700); λ_{min} 235; pH 12: λ_{max} 283 (11700); λ_{min} 244; ε260 (M) 12010. δ_C (CD_3OD) 164.0, 151.9, 148.8, 141.9, 138.1, 118.9, 119.1, 89.2, 86.5, 74.6, 71.2, 62.1, 54.8. ^{1}H NMR showed a 4:1 ratio of imino-amino tautomers.

Major tautomer. δ_H 11.23 (1H, d, *J* 3.5, NH), 8.08 (1H, s, H8), 7.58 (1H, d, *J* 3.5, H2), 6.00 (1H, m, $OCH_2C\mathit{H}$=CH_2), 5.73 (1H, d, *J* 5.9, 1′-H), 5.44 (1H, d, *J* 6.2, 2′-OH), 5.35 and 5.29 (2× 1H, 2× dd, *J* 3.4, 1.6, CH=CH_2), 5.17 (1H, m, 3′-OH), 5.08 (1H, t, *J* 5.9, 5′-OH), 4.47 (2H, dd, *J* 6.2, 1.3, OCH_2), 4.41 (2H, dd, *J* 11.2, 5.9, 2′-H), 4.07 (1H, app dd, *J* 8.3, 4.8, 3′-H), 3.89 (1H, app dd, *J* 7.2, 3.7, 4′-H), 3.60 and 3.50 (2× 1H, 2× m, 5′-H_a and 5′-H_b).

Minor tautomer. δ_H 11.00 (1H, br s, NH), 8.45 (1H, s, H8), 8.29 (1H, s, H2), 6.00 (1H, m, $OCH_2C\mathit{H}$=CH_2), 5.90 (1H, d, *J* 5.3, 1′-H), 5.47 (1H, d, *J* 6.2, 2′-OH), 5.35 and 5.29 (2× 1H, 2× m, CH=CH_2), 5.19 (1H, m, 3′-OH), 5.15 (1H, t, *J* 5.9, 5′-OH), 4.58 (2H, dd, *J* 11.0, 5.7, 2′-H), 4.47 (2H, m, OCH_2), 4.11 (1H, app dd, *J* 9.8, 4.8, 3′-H), 3.94 (1H, m, 4′-H), 3.60 and 3.50 (2× 1H, 2× m, 5′-H_a and 5′-H_b).

5.4. In vitro parasite growth inhibition assays

In vitro parasite growth inhibition was assessed by the incorporation of [^{3}H] hypoxanthine based on the method used by Desjardins[42] and modified as described.[43] All assays included chloroquine diphosphate as a standard

and control wells with untreated infected and uninfected erythrocytes. The compounds were dissolved in 100% dimethylsulfoxide (Sigma) and serial dilutions were made in assay medium. Fifty microlitres of *P. falciparum* (65–75% ring stage) culture at 0.5% parasitaemia or uninfected erythrocytes were added to each well reaching a final volume of 100 μL per well, a final haematocrit of 2.5% and final dimethylsulfoxide concentrations $\leqslant$0.01%. Plates were incubated at 37 °C in 5% CO_2, 95% air mixture for 24 h, at which point 20 μL (0.1 μCi/well) of [^{3}H] hypoxanthine (Perkin-Elmer, Hounslow, UK) was added to each well and returned to the incubator for an additional 24 h incubation period at which point, the experiment was terminated by placing the plates in a −80 °C freezer. Plates were thawed and harvested onto glass fibre filter mats using a 96-well cell harvester (Harvester 96™, Tomtec, Oxon, UK) and left to dry. After the addition of MeltiLex™ solid scintillant (Perkin-Elmer, Hounslow, UK) the incorporated radioactivity was counted using a 1450 Betalux scintillation counter (Wallac®). Data acquired were exported into an Excel spreadsheet, and the IC_{50}/IC_{90} values of each drug were calculated by using XLFit® (ID Business Solutions Ltd, UK) line fitting software.

5.5. In vivo full suppressive 4-day Peters' test

In vivo tests were performed under the Home Office Animals (Scientific Procedures) Act 1986. CD-1 outbred 20 g male mice (Charles Rivers, UK) were kept in specific pathogen-free conditions and fed ad libitum. For subcutaneous administration, the compounds were dissolved in 10% dimethylsulfoxide (DMSO) and 0.05% Tween 80 (Sigma, Dorset, UK) in distilled water. For oral administration, compounds were dissolved in standard suspending formula (SSV) [0.5% sodium carboxymethylcellulose, 0.5% benzyl alcohol, 0.4% Tween 80, 0.9% NaCl (all Sigma)]. For intravenous administration, compounds were dissolved in 5% dextrose. Mice were infected intravenously with 2×10^6 *P. berghei* AS parasitized red cells and treated intravenously (iv), subcutaneously (sc) or orally (po) with 0.2 mL of a solution of the test compounds two hours (day 0) and on days 1, 2, 3 and 4 post-infection, at a dose of 30 mg test compound per kg body weight. Parasitaemia was determined by microscopic examination of Giemsa stained blood films taken on day 5 post-infection. Microscopic counts of blood films from each mouse were processed using MICROSOFT® EXCEL (Microsoft Corp.) and expressed as percentages of inhibition from the arithmetic mean parasitaemias of each group in relation to the untreated group.

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